Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1. (Original) A method for engineering a cell to produce an increased amount of hydrogen comprising:

(a) providing a mutagenized nucleic acid sequence derived from a first gene that encodes a protein involved in a hydrogen production pathway;

(b) transforming a cell with said mutagenized nucleic acid sequence; and

(c) screening or selecting the cell for an increased amount of hydrogen.

2. (Original) The method of claim 1, wherein a plurality of mutagenized nucleic acid sequences are used to transform a population of cells, followed by the screening or selecting.

3. (Original) The method of claim 1, wherein the first gene is selected from the group that encodes ferredoxin, catalase, isoamylase, malate dehydrogenase, 14-3-3 protein, enolase, aldolase, ribosomal protein S8, ribosomal protein L17, ribosomal protein S18, ribosomal protein L37, ribosomal protein L12, ribosomal protein S15, iron-hydrogenase, nickel-iron hydrogenase, and components of the photosystem I, photosystem II, light harvesting antenna and cytochrome b_6-f complexes.

4. (Original) The method of claim 3, wherein the first gene encodes an iron-hydrogenase.

5. (Original) The method of claim 4, wherein at least one amino acid from the segment $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ or the segment ADX^8TIX^9EE is substituted by a different amino acid in the protein encoded by the first gene to generate the mutagenized nucleic acid sequence.

6. (Original) The method of claim 5, wherein the mutagenized nucleic acid sequence is generated by gene reassembly.

7. (Original) The method of claim 5, wherein the mutagenized nucleic acid sequence is generated by site-directed mutagenesis.

8. (Original) The method of claim 5, wherein an amino acid that is substituted for the at least one amino acid has a side chain of higher molecular weight than the side chain of the at least one amino acid.

9. (Original) The method of claim 5, wherein saturation mutagenesis is performed on the at least one amino acid.

10. (Currently amended) The method of claim 5, wherein the mutagenized nucleic acid sequence is generated by a mutagenesis method described in U.S. Patents selected from the group consisting of ~~5,537,776;~~ 5,965,408; 6,171,820; 6,174,673; 6,238,884; 6,326,204; 6,344,328; 6,352,842; 6,358,709; 6361,97; 6,368,798; 6,440,668; 6537,776; and 6,605,449.

11. (Original) The method of claim 6, wherein the gene reassembly is performed using nucleic acid molecules that encode proteins of SEQ ID NOs: 1-112 or segments thereof.

12. (Original) The method of claim 4, wherein the mutagenized nucleic acid sequence encodes an iron hydrogenase protein that functionally interacts with a ferredoxin protein in the cell.

13. (Original) The method of claim 1, wherein the screening or selecting occurs in the presence of oxygen at a concentration selected from the ranges comprising more than 0.5%, more than 5.0%, more than 10%, more than 15%, approximately 21%, more than 21%, more than 25%, more than 30% or more than 35% oxygen.

14. (Original) The method of claim 1, wherein the mutagenized nucleic acid sequence is operably linked to a promoter that is activated by light.

15. (Original) The method of claim 1, wherein the mutagenized nucleic acid sequence is generated by gene reassembly.

16. (Original) The method of claim 1, wherein the cell is a green algae species.

17. (Original) The method of claim 1, wherein cell is of the genus Chlamydomonas.

18. (Original) The method of claim 1, further comprising the steps of;

(a) identifying a first independent transformant which produces an increased amount of hydrogen from step (c) of claim 1;

(b) recovering the mutagenized nucleic acid sequence from the independent transformant;

(c) further mutagenizing the recovered mutagenized nucleic acid sequence to create a new library of mutagenized nucleic acid sequences;

(d) transforming cells with the new library of mutagenized nucleic acid sequences; and

(e) screening or selecting for a new independent transformant from the new library that generates an increased amount of hydrogen compared to the first independent transformant.

19. (Currently amended) The method of claim 18 wherein the mutagenized nucleic acid ~~sequencs~~ sequences are generated by gene reassembly.

20. (Original) The method of claim 18, wherein a plurality of mutagenized nucleic acid sequences are recovered from a plurality of independent transformants which produce an increased amount of hydrogen from step (c) of claim 1, and wherein the plurality of mutagenized nucleic acid sequences are subjected to gene reassembly to generate the new library.

21. (Original) The method of claim 1, wherein the screening or selecting occurs by culturing cells in liquid growth media.

22. (Original) The method of claim 21, wherein the growth media is a photoautotrophic growth-requiring minimal media.

23. (Original) The method of claim 1, wherein the screening or selecting occurs in a non-transparent culture container.

24. (Original) A method according to claim 1, wherein the mutagenized nucleic acid sequence is operably linked to a promoter that is constitutively activated.

25. (Original) The method of claim 15, wherein the mutagenized nucleic acid sequence is obtained by subjecting nucleic acid sequences that encode proteins that are expressed when cells are exposed to conditions more conducive to the generation of hydrogen to gene reassembly, wherein the proteins are naturally encoded by genes in organisms from more than one species.

26. (Original) The method of claim 19, wherein the proteins are iron hydrogenases or nickel-iron hydrogenases.

27. (Original) The method of claim 1, further comprising repeating the steps of claim 1 using a second gene distinct from the first gene.

28. (Original) The method of claim 27, further comprising:

(a) mating at least one cell of a strain containing a mutagenized form of the first gene:

i. wherein the at least one cell is identified by the screening or selecting; or

ii. wherein the at least one cell is derived through mating from a cell identified by the screening or selecting;

to at least one cell of a distinct strain containing a mutagenized form of the second gene:

iii. wherein the at least one cell is identified by the screening or selecting; or

iv. wherein the at least one cell is derived through mating from a cell identified by the screening or selecting; and

(b) screening or selecting for a progeny cell that produces an increased amount of hydrogen compared to any parent cell.

29. (Withdrawn) A method of hydrogen production, comprising:

(a) placing cell containing a mutagenized nucleic acid sequence corresponding to a gene that is involved in a hydrogen production pathway into liquid culture media or on to solid culture media, wherein the mutagenized nucleic acid sequence is operably linked to a transcriptional promoter sequence;

(b) culturing said transformed cell under conditions sufficient to stimulate transcription of said mutagenized nucleic acid sequence(s); and

(c) collecting an evolved gas.

30. (Withdrawn) The method of claim 29, wherein the culture media is photoautotrophic growth requiring media.

31. (Withdrawn) A method of multiparental mating of microbes that mate in response to a stimulus, comprising:

(a) providing a cell from each of 3 or more strains of microbes capable of mating to each other in culture medium;

(b) providing the stimulus;

(c) allowing cells to mate and produce progeny;

(d) allowing the progeny cells to achieve sexual reproduction capability;

(e) providing the stimulus at least one more time; and

(f) screening or selecting the further progeny for a desired phenotype.

32. (Withdrawn) The method of claim 31, wherein the microbes are green algae and the stimulus is the removal of nitrogen from the media and illumination by light comprising a wavelength between about 0.42-0.52 micrometers.

33. (Withdrawn) The method of claim 32, wherein the green algae are of the Chlamydomonas genus.

34. (Withdrawn) The method of claim 33, wherein the species is selected from the group comprising reinhardtii, eugametos, incerta, and moewusii.

35. (Withdrawn) The method of claim 31, wherein the stimulus is interruption of exponential growth in continuous light with a reduction in light, followed by addition of light.

36. (Withdrawn) The method of claim 35, wherein the reduction in light occurs for a period selected from the group consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more than 12 hours.

37~~6~~. (Currently amended and Withdrawn) The method of claim 31, wherein the microbes are of the Scendesmus genus and the stimulus is the addition of chromium to the culture media.

38~~7~~. (Currently amended and Withdrawn) The method of claim 31, wherein the desired phenotype is hydrogen production.

39~~8~~. (Currently amended and Withdrawn) The method of claim 31, wherein nucleic acid exchange occurs between only two parental cells at a time during the mating process.

40. (New) The method of claim 4, wherein at least one amino acid from the segment $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ and at least one amino acid from the segment ADX^8TIX^9EE are both substituted by a different amino acid in the protein encoded by the first gene in a gene reassembly reaction to generate the mutagenized nucleic acid sequence.

41. (New) The method of claim 40, wherein more than one nucleic acid encoding at least a portion of the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ segment and more than one nucleic acid encoding at least a portion of the ADX^8TIX^9EE segment are placed in the gene reassembly

reaction, wherein the more than one nucleic acid sequences encoding at least a portion of each segment contain distinct nucleotide sequences.